Performance of the Angio Detect™ in-clinic test kit for detection of Angiostrongylus vasorum infection in dog samples from Europe

Liu, Jiayou ; Schnyder, Manuela ; Willesen, Jakob L ; Potter, Adam ; Chandrasekharan, Ramaswamy

Abstract: Angiostrongylosis is a crucial differential diagnosis in any dog with respiratory, bleeding, neurological, or syncopal signs of unknown etiology in endemic areas. Many cases of angiostrongylosis have a fatal outcome; subclinical angiostrongylosis also has been reported. The most common method for supporting diagnosis of angiostrongylosis has been identification of Angiostrongylus vasorum first stage larvae in the feces by the Baermann-Wetzel method. Although considered as gold standard, this method has technical and sampling challenges and cannot detect infections during prepatency or in case of intermittent shedding of the larvae. A rapid in-clinic antigen test has been developed for serologic detection of A. vasorum infections using blood samples (Angio Detect™, IDEXX Laboratories Inc., Westbrook, Maine, USA). The study reported here was conducted to determine diagnostic sensitivity and specificity of the Angio Detect test kit by comparing Angio Detect testing results using serum or plasma samples with the results of Baermann-Wetzel testing using matched fecal samples. Samples from 214 dogs [with clinically suspected (N = 195) or diagnosed angiostrongylosis (N = 19)] were used for this evaluation. Baermann-Wetzel testing was performed independently at commercial reference laboratories or at university hospitals. All serum/plasma samples were blinded and randomized before testing with Angio Detect. The Angio Detect test was positive for 34 of the 35 cases found positive by the Baermann-Wetzel method; sensitivity of the Angio Detect test was 97.1% (95%CI: 85.1%–99.9%). The Angio Detect test was negative for 177 of 179 samples that were negative by the Baermann-Wetzel test; specificity was 98.9% (95%CI: 96.0%–99.9%). In cross-reactivity testing, all 89 samples from dogs confirmed to be infected with other common nematodes (Dirofilaria immitis, D. repens, Crenosoma vulpis, hookworms, ascarids, or whipworms) were all negative for A. vasorum by the Angio Detect antigen test. Angio Detect provides a rapid and reliable method for diagnosis of A. vasorum in clinically suspected dogs at risk for infection. The test requires minimal steps by the operator and provides results in 15 min, allowing the clinician to initiate treatment for positive dogs before leaving the clinic.

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Original Article

Performance of the Angio Detect™ in-clinic test kit for detection of *Angiostrongylus vasorum* infection in dog samples from Europe

Jiayou Liu [a], Manuela Schnyder [b], Jakob L. Willesen [c], Adam Potter [a], Ramaswamy Chandrashekar [a,*]

[a] IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, ME, 04092, USA
[b] Institute of Parasitology, Vetsuisse Faculty, Winterhurerstrasse 266a, Zurich 8057, Switzerland
[c] Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical, University of Copenhagen, 16 Dyrlaegevej, 1870 Frb. C., Denmark

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ABSTRACT

Angiostrongylosis is a crucial differential diagnosis in any dog with respiratory, bleeding, neurological, or syncopal signs of unknown etiology in endemic areas. Many cases of angiostrongylosis have a fatal outcome; subclinical angiostrongylosis also has been reported. The most common method for supporting diagnosis of angiostrongylosis has been identification of *Angiostrongylus vasorum* first stage larvae in the feces by the Baermann-Wetzel method. Although considered as gold standard, this method has technical and sampling challenges and cannot detect infections during prepatency or in case of intermittent shedding of the larvae. A rapid in-clinic antigen test has been developed for serologic detection of *A. vasorum* infections using blood samples (Angio Detect™, IDEXX Laboratories Inc., Westbrook, Maine, USA). The study reported here was conducted to determine diagnostic sensitivity and specificity of the Angio Detect test kit by comparing Angio Detect testing results using serum or plasma samples with the results of Baermann-Wetzel testing using matched fecal samples. Samples from 214 dogs [with clinically suspected (N = 195) or diagnosed angiostrongylosis (N = 19)] were used for this evaluation. Baermann-Wetzel testing was performed independently at commercial reference laboratories or at university hospitals. All serum/plasma samples were blinded and randomized before testing with Angio Detect. The Angio Detect test was positive for 34 of the 35 cases found positive by the Baermann-Wetzel method; sensitivity of the Angio Detect test was 97.1% (95%CI: 85.1%–99.9%). The Angio Detect test was negative for 177 of 179 samples that were negative by the Baermann-Wetzel test; specificity was 98.9% (95%CI: 96.0%–99.9%). In cross-reactivity testing, all 89 samples from dogs confirmed to be infected with other common nematodes (*Dirofilaria immitis*, *D. repens*, *Crenosoma vulpis*, hookworms, ascarids, or whipworms) were all negative for *A. vasorum* by the Angio Detect antigen test. Angio Detect provides a rapid and reliable method for diagnosis of *A. vasorum* in clinically suspected dogs at risk for infection. The test requires minimal steps by the operator and provides results in 15 min, allowing the clinician to initiate treatment for positive dogs before leaving the clinic.

1. Introduction

Angiostrongylosis is a disease caused by the metastrongylid nematode *Angiostrongylus vasorum*, which has been referred to as the "French Heartworm" reflecting its first recorded incidence in France (Serres, 1854). Canine angiostrongylosis is considered endemic in several areas of Europe, as well as in Brazil (Lima et al., 1994) in South America, and in Uganda (Bwangamoi, 1972) in Africa. It is also found in parts of North America (Conboy, 2004; Kistler et al., 2014), and its distribution is increasingly reported both in particularly in foxes and in dogs. The estimated prevalence among the dog populations in the United Kingdom, Denmark, Germany and Greece ranges from 0.3% to 9.8% (Elsheikha et al., 2014). Foxes are considered an important reservoir of *A. vasorum* and the estimated prevalence in fox populations ranges from 5% to 56% (Koch and Willesen, 2009), and as high as 80% in Zealand, Denmark (Al-Sabi et al., 2014).

The adult stage of *A. vasorum* lives in the pulmonary arteries and right atrium of dogs and other canids. In the definitive host, the adults produce eggs that hatch into first-stage larvae (L1), which penetrate the alveoli, migrate up to the oropharynx, get swallowed by the host before being eliminated in the feces. These first-stage larvae infect a snail or slug as an intermediate host and develop within 10 to 16 days to the third stage infective larvae (L3) (Guilhon and Bressou, 1960). Dogs are suspected to be infected by ingestion of the gastropod or frogs, which may serve as paratenic hosts (Bolt et al., 1993). The L3 penetrate

* Corresponding author at: IDEXX Laboratories, Westbrook, ME 04092, USA.
E-mail address: Ramaswamy-Chandrashekar@idexx.com (R. Chandrashekar).

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the gut wall and eventually enter the portal circulation to reach the right atrium and pulmonary arteries. Generally, the prepatent period ranges from 38 to 57 days (Guilhon and Cens, 1973).

Clinical signs of infection are highly variable. The infected dogs may present with respiratory symptoms including cough, dyspnea, and other respiratory and cardiovascular signs; coagulopathies and neurologic dysfunctions may be present as the disease progresses (Chapman et al., 2004; Koch and Willesen, 2009; Schnyder et al., 2010). Considering the challenge to differentiate some of the clinical signs of *A. vasorum* infections and the subtle course of the pathological process, many cases of angiostrongylosis have a fatal outcome (Chapman et al., 2004; Staebler et al., 2005; Wessmann et al., 2006), making early and accurate diagnosis critical.

The most common method for diagnosing *A. vasorum* infection has been the Baermann-Wetzel method (Deplazes et al., 2016), which is used to isolate and microscopically identify L1 excreted in the feces. While generally considered as the reference standard, this technique has several important limitations. Due to intermittent larval shedding (Oliveira-Junior et al., 2006), it requires dog owners collect fresh feces for three consecutive days to improve sensitivity (Koch and Willesen, 2009). Compliance is often weak in practice. It also requires skills to differentiate larvae of various species (which in particular cases can be supported by PCR analyses). For this reason, samples are frequently submitted to a reference laboratory, which can delay definitive diagnosis.

Over a period of several years, studies to identify and detect circulating antigens have led to the development of various test methods for detection of *A. vasorum* infections in dogs (Verzberger-Epshtein et al., 2008). Most recently, Schnyder et al. (2011) developed a sensitive and specific ELISA using species-specific monoclonal antibodies. The specific monoclonal antibodies were used to develop the in-clinic test. In this study, we evaluate the performance of Angio Detect with samples from client-owned sick dogs suspected or clinically diagnosed of angiostrongylosis.

2. Methods

In a special study that was conducted, between June 2012 and April 2013, veterinarians in the UK were asked to submit samples for Baermann testing from dogs suspected of angiostrongylosis. Sick dogs were selected at the sole discretion of the attending veterinarians on a voluntary basis. Matched fecal and serum or plasma samples were obtained concurrently from a total of 195 dogs. The fecal samples were analysed immediately using the Baermann-Wetzel method (Deplazes et al., 2016), by commercial laboratories (IDEXX Reference Laboratories, Inc.); the matched serum or plasma samples were kept frozen at −20 °C until they were tested by Angio Detect as described below.

Archived serum samples from 19 dogs diagnosed with angiostrongylosis were also included in this study. Clinical diagnosis and confirmation by the Baermann-Wetzel fecal analysis were performed by authors MS and JLW in their university clinics in Zurich, Switzerland and Copenhagen, Denmark, respectively.

Additional negative control samples from 89 dogs were included for evaluation of specificity and cross reactivity. Among them were 79 dogs originating from the US and positive for *Dirofilaria immitis* (confirmed by necropsy) and negative for *A. vasorum* by a sensitive and specific ELISA antigen test (Schnyder et al., 2011), 3 dogs positive for *Crenosoma vulpis* and negative for *A. vasorum* (confirmed by Baermann-Wetzel technique), 4 dogs experimentally infected with *Dirofilaria repens*, and 3 dogs each experimentally infected with hookworms, ascarids, and whipworms.

All serum or plasma samples were combined into a single set, randomized, and blind-labelled before testing with Angio Detect. The persons who conducted the testing were blinded as to the status of each sample. The samples were tested with Angio Detect according to the manufacturer's instructions.

Samples yielding discrepant results between the Baermann-Wetzel test and the Angio Detect test were further evaluated by the ELISA-based antigen test (Schnyder et al., 2011).

3. Results

From 195 dogs with suspected angiostrongylosis, the Baermann-Wetzel analysis identified 16 dogs positive for *A. vasorum* L1 in the feces while 179 were negative. Of the 16 fecal-positive dogs, 15 tested positive and one negative for circulating antigen by Angio Detect. The one discrepant sample was tested negative by previously described ELISA antigen test (Schnyder et al., 2011). Of the 179 serum or plasma samples from dogs testing negative for *A. vasorum* L1 by the Baermann-Wetzel fecal analysis, 177 tested negative and two positive for circulating antigen by Angio Detect. The two discrepant samples were tested positive by the ELISA antigen test (Schnyder et al., 2014).

Of archived serum samples from Baermann-confirmed clinical cases, all 19 samples were tested positive for circulating antigen by Angio Detect. Combining these two sample sets (35 Baermann positive and 179 Baermann negative), Angio Detect demonstrated an overall sensitivity of 97.1% (95%CI: 85.1%–99.9%), and a specificity of 98.9% (95%CI: 96.0%–99.9%) (Table 1), compared to the Baermann-Wetzel analysis.

In the cross-reactivity testing, all 89 samples from dogs confirmed to be infected with *D. immitis*, *C. vulpis*, *D. repens*, hookworms, ascarids, or whipworms, were negative for *A. vasorum* by the Angio Detect test.

4. Discussion

Angio Detect is a rapid in clinic test designed to detect circulating antigen based on *A. vasorum* specific antibodies (Schnyder et al., 2014). In the presented study, Angio Detect was found to have a sensitivity of 97.1% (95%CI: 85.1%–99.9%) and a specificity of 98.9% (95%CI: 96.0%–99.9%) compared to Baermann-Wetzel analysis. Similar results were reported in a previous study that used samples from both clinical and experimental infected samples (Schnyder et al., 2014). Relatively higher sensitivity of Angio Detect found in this study may be attributable to the specific sample set evaluated. This sample set was limited to client-owned sick dogs and to previously confirmed clinical cases. They may represent dogs at a later stage of infection when outward clinical manifestations were already noticed by the animal owners. Using dogs experimentally infected with *A. vasorum*, Schnyder et al. (2014) demonstrated that the Angio Detect test was 100% sensitive at 14 weeks post inoculation and was positive in some cases as early as 9 weeks.

It is generally recommended to perform Baermann-Wetzel analysis with fresh fecal samples collected over three days to improve sensitivity (Koch and Willesen, 2009). However, this recommendation is frequently not followed in practice due to lack of compliance for appropriate sample collection. Also for our study with suspected cases, fecal samples from multiple days were rarely submitted. It is then likely that we didn't achieve the highest possible sensitivity for Baermann-Wetzel analysis due to these sampling limitations. Unlike Baermann analysis of feces, detection of *A. vasorum* circulating antigen is not affected by intermittent shedding of L1. This may explain the results of two dogs, positive

Table 1
Comparison of IDEXX Angio Detect™ *Angiostrongylus vasorum* antigen test with conventional fecal test (Baermann-Wetzel) results in dogs with suspected or confirmed angiostrongylosis (n = 214).

Test	Baermann test/Angio Detect		
Sensitivity	Pos/Pos	Neg/Pos	Relative sensitivity (95%CI)
	35/34	0/1	97.1% (85.1%–99.9%)
Specificity	Pos/Neg	Neg/Neg	Relative specificity (95%CI)
	0/2	179/177	98.9% (96.0%–99.9%)

CI, confidence interval; Neg, negative; Pos, positive.

by both Angio Detect and confirmatory ELISA, but negative by Baermann analysis.

Based on the results of this study, Angio Detect can be used as a first line test for dogs displaying clinical signs of angiostrongylosis, especially in emergency situations (Chapman et al., 2004; Wessmann et al., 2006; Gredal et al., 2011), where the parasite is endemic and dogs are at risk of infection. The test also can be used for monitoring dogs post anthelminthic treatments. In a recent study, *A. vasorum* infected dogs became negative with the Angio Detect test within three to seven weeks after anthelminthic treatment (Schnyder et al., 2014).

While the Angio Detect antigen test is highly accurate with clinical cases, a negative Angio Detect result alone should not be used to rule out angiostrongylosis, if clinical suspicion of *A. vasorum* infections or infections with other lungworms such as *Crenosoma vulpis* is still high. If performed with fresh fecal samples, Baermann analysis can detect *A. vasorum* positive dogs as early as 6 weeks after infection, and the recommended collection of feces over three days may additionally increase its sensitivity, overcoming intermittent larval shedding. There also might be cases where blood sampling does not come into consideration while fecal samples are readily available. It is advisable that Baermann remains a test supplementing the overall diagnostic approach to lungworm diagnosis.

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Authors Liu, Potter and Chandrashekar are employees of IDEXX Laboratories. Drs. Schnyder and Willesen are study collaborators and have no conflict of interest.

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